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(54) Title: CONJUGATES OF ANTI-IDIOTYPE ANTIBODIES AND CARRIERS AND THEIR USE IN EPITOPE-DIRECTED IMMUNIZATION

(57) Abstract

Disclosed are methods and products for epitope-directed immunization with a vaccine including an anti-idiotype antibody. The anti-idiotype antibody is conjutated to a carrier. The carrier is a protein against which the vaccine recipient has previously been immunized or otherwise previously exposed, or a peptide segment of such a protein containing determinants recognized by the antigen-specific receptors on the T helper cells, or an antibody which enhances the immune response against the anti-idiotype. The carrier should not alter the conformation of the anti-idiotype after conjugation. The anti-idiotype antibody is preferably one which induces production of neutralizing antibodies against HIV-1, in which case the preferred carrier is HBsAg or HIV-1 p24, or a T helper peptide derived from HBsAg or HIV-1 p24. It is more preferable if the anti-idiotype antibody induces production of neutralizing antibodies against the external envelope glycoprotein gp120 of HIV-1, and it is most preferable if it induces them against the principle neutralizing determinant of gp120. One such exemplary anti-idiotype antibody is AB19-4.

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CONJUGATES OF ANTI-IDIOTYPE ANTIBODIES AND CARRIERS AND THEIR USE IN EPITOPE-DIRECTED IMMUNIZATION

Field of the Invention

The invention relates to epitope-directed immunization with a vaccine in which an anti-idiotype antibody (or a fragment thereof) specific for a pathogen or allergen is conjugated to a carrier, and wherein the carrier is one against which the vaccine recipient has previously been immunized or otherwise previously exposed, or it is a carrier which enhances the immune response against the anti-idiotype.

Background of the Invention

It is fundamental that the introduction of a foreign antigen to an animal causes an immune response, including the production of antibodies (Ab1) specific for that antigen. It is also well recognized that anti-idiotype antibodies (Ab2) to the paratope of Ab1, produced by immunizing with Ab1, bear the internal image of the antigenic determinant which Ab1 recognizes. As a result, immunization with Ab2 can elicit *in vivo* the production of another set of antibodies (Ab3) which, like Ab1, bind to the antigen which was originally introduced. If the antigen originally introduced is a pathogen or allergen or a potential pathogen or allergen, immunization with Ab2 will actively protect against that substance.

The Ab2 are, therefore, useful as vaccines, because they induce production of endogenous Ab3.

Vaccination (or "active immunization") is an effective means to stop the spread of the deadly disease AIDS, or Acquired Immune Deficiency Syndrome. Without an effective vaccine, the disease may increase to epidemic proportions in the general population. Anti-idiotype antibodies have been suggested for use in active immunization against human immunodeficiency virus-type 1 ("HIV-1"), the causative agent of AIDS. *See* U.S. Patent Application Serial No. 07/454,161, filed December 21, 1989.

One requirement for active immunization with Ab2 is that the conformational structure of the antigen binding site of the Ab2 should be preserved during the immunization. If the conformation changes, the Ab2 will no longer bear the internal image of the antigenic determinant which Ab1 recognizes, and will not induce production of antigen-specific Ab3.

In conventional active immunization the immunogen may be a protein subunit or fixed, deactivated virus particles, rather than an anti-idiotype antibody. In such conventional immunization, an adjuvant is often used to augment the immune response to the immunogen. Suitable adjuvants may include aluminum compounds, liposomes, synthetic polymers, Freund's

incomplete adjuvant, muramyl dipeptides, and monokines and lymphokines. *See* Warren, H.S. *et al., Ann. Rev. Immunol.* 4:369-88 (1986).

Many of these adjuvants function by activating the accessory antigen-presenting macrophages at the sites of injection. They also help to retain the immunogens and release them over long periods. As a result, they help generate higher titers of anti-immunogen antibodies in the blood.

Where the immunogen in the active immunization is an anti-idiotype antibody, many adjuvants are not well-suited. A number of adjuvants can change the conformational structure of the anti-idiotype's antigen binding site, including those which lower the pH, *e.g.*, some alum preparations, or those having an oil phase, *e.g.*, Freund's adjuvants. Thus, a narrower selection of adjuvants are suitable for use with an anti-idiotype vaccine than with a protein subunit or a viral antigen.

Rather than using adjuvants, which may have the limitations noted above, another method to enhance the immunogenicity of an anti-idiotype is based on the so-called "carrier effect." It is known that where the immunogen is a potential antigen (or "hapten") conjugated to a carrier protein, and memory helper T cells already exist which recognize the carrier protein, this enhances production

of antibodies against the hapten. These helper T cells bind to certain epitopes on the carrier protein, and leave the hapten's epitopes exposed so that resting, antigen-specific B cells can bind to the hapten. These resting B cells will then become activated, undergoing clonal expansion, and will mature into plasma cells which secrete antibodies against the hapten. This carrier effect, which underlies the fundamental mechanism of T cell-B cell cooperation in immune response, has also been shown to occur when the carrier is a peptide segment (hereinafter "the T helper peptide") containing determinants recognized by the antigen-specific receptors on the T helper cells.

Thus, when immunization is with such a carrier/anti-idiotype conjugate, where the subject has been previously immunized or otherwise exposed to the carrier, helper T cells will recognize the carrier. This will leave the immunogenic sites on the anti-idiotype exposed and available for binding by resting, antigen-specific B cells. These resting B cells will become activated, undergoing clonal expansion, and then will mature into plasma cells which secrete antibody (Ab3) against the anti-idiotype. More antibody against the anti-idiotype is likely to be produced than when the anti-idiotype alone is used as the vaccine.

Summary of the Invention

The invention includes epitope-directed immunization with a vaccine in which an anti-idiotype antibody is conjugated to a carrier, which can be either a protein or its derived T helper peptide. The carrier is one against which the vaccine recipient has previously been immunized or otherwise previously exposed, or which enhances the immune response against the anti-idiotype antibody. The carrier should not alter the conformation of the anti-idiotype after conjugation.

The anti-idiotype antibody is preferably one which induces production of neutralizing antibodies against HIV-1, such as a carrier/anti-idiotype conjugate which induces production of neutralizing antibodies (Ab3) against the external envelope glycoprotein, gp120, of HIV-1, against the principal neutralizing determinant ("PND") of gp120, or against another neutralizing domain of HIV-1. One exemplary anti-idiotype antibody which induces antibodies against the PND is AB19-4. The cell line which produces AB19-4 is currently on deposit at the American Type Culture Collection ("ATCC"), Rockville, Maryland, under Accession No. HB 10315.

Where the anti-idiotype induces Ab3 against HIV-1, the carrier preferably is HBsAg or HIV-1 p24, or a peptide of either

HBsAg or HIV-1 p24 including a T helper determinant. It is known that many individuals in the high risk groups for HIV-1 infection are also at high risk for hepatitis B virus infection. Many people in these groups have already been vaccinated with the hepatitis B virus vaccine using hepatitis B surface antigen (HBsAg). Thus, HBsAg (and related T helper peptides) is a preferred carrier for conjugation with an anti-idiotype antibody which induces production of HIV-1 neutralizing antibodies.

For individuals previously exposed to HIV-1, the p24 peptide of HIV-1 is suitable for conjugation with an anti-idiotype antibody which induces HIV-1 neutralizing antibodies. The p24 peptide of HIV-1 is very conserved among different strains and isolates of HIV-1, and induces a vigorous immune response. Provided that their immune systems are not yet seriously compromised, persons previously exposed to HIV-1 will have memory helper T cells against this p24 peptide. Administration of a conjugate of this peptide and AB19-4 will cause the memory helper T cells to bind to this p24 peptide, resulting in activation of resting B cells, and ultimately in increased secretion of HIV-1 neutralizing Ab3.

This p24/anti-idiotype conjugate, when administered to people exposed to HIV-1 before their immune systems are

seriously compromised, will probably result in increased secretion of HIV-1 neutralizing Ab3. These Ab3 can lower the viral titer to the point where remaining virus can be eliminated by the body's immune defense mechanisms.

A conjugate of HBsAg or p24 of HIV-1 (or a T helper peptide derived from HBsAg or p24) with AB19-4 is useful for HIV-1-infected individuals who produce some antibodies against gp120, but produce antibodies specific for the PND at a low level. The epitope-directed immunization with an AB19-4 conjugate will induce specifically the production of Ab3 against the PND. The induced PND-specific Ab3 may inhibit HIV-1 replication in HIV-1-infected but asymptomatic individuals and prevent disease progression.

Monoclonal anti-idiotype antibodies, such as AB19-4, are initially murine derived. The conjugates of the invention also include conjugates of human (or humanized) anti-idiotypes, or fragments of human anti-idiotypes, with carriers.

The carriers of the invention can also include substances which induce an enhanced immune response against the anti-idiotype antibody. Such substances include anti-*migis-δ* and anti-*migis-μ* antibodies, which specifically target the extracellular segments of membrane-bound IgD and IgM, respectively, and are

further described in published International application Nos. PCT/US88/04706; PCT/US90/03532; PCT/US90/05229.

The invention will now be described in greater detail with specific reference to the method of making and using it.

Detailed Description of the Preferred Embodiments

Manner and Process of Making and Using the Invention

As noted above, the invention includes carrier/anti-idiotype conjugates and their use in epitope-directed active immunization. The method of making and using this conjugate is discussed below.

Isolating an anti-idiotype antibody (Ab2) suitable for use with the conjugates of the invention involves several steps. First, a monoclonal antibody (Ab1) against the target antigen must be produced. Using Ab1 as an immunogen, paratope-specific Ab2, which bear the internal image of the original antigen, are produced.

The Ab2 can be screened based on their binding to Ab1 and the extent to which the original antigen can compete with such binding. The paratope-specific Ab2 are further screened based on their ability to induce, *in vivo*, Ab3 which, like the original Ab1, bind to the antigen.

After being produced and screened, the Ab2 is conjugated to a carrier (which is a suitable protein or peptide) to

form a conjugate of the invention. The method of conjugation is very well established. Cross-linkers such as glutaraldehyde, or bis (sulfosuccinimidyl) suberate and preferably disulfosuccinimidyl tartarate, both available from Pierce Chemical Co., Rockford, IL (Catalogue Nos. 21579, 20591) can be used.

Rather than chemically conjugating the carrier with Ab2, the carrier peptide and the heavy or the light chain of Ab2 can be produced as a contiguous peptide by expressing a composite gene containing the DNA segment encoding the heavy or light chain and the DNA segment encoding the carrier peptide. This molecular biological method will be most appropriate for expressing shortened heavy or light chains, *e.g.*, the variable regions, and the T helper peptide segments of the carrier protein.

One preferred target antigen for Ab1 is the PND on the external envelope glycoprotein gp120 of HIV-1. The PND is the peptide segment on gp120 from amino acid residue numbers 296 to 331, as determined from the gp120 sequence of the HTLV-III_B. *See* Devash, Y., *Proc. Nat'l. Acad. Sci. USA* 87:3445-3449 (1990). The PND peptide segment is in the relatively variable region, V3, of gp120. However, recent studies indicate that there are conserved features in the PND segment. The amino acid sequences of PND segments in field HIV-1 isolates from patients

are closely related. *See* LaRosa, G.J. *et. al.*, *Science* 249:932-935 (1990). Antibodies which target the PND are generally effective in neutralizing HIV-1 infection. One example of an Ab1 which targets the PND and neutralizes HIV-1 is BAT123, which is described fully in U.S. Patent Application Serial No. 07/137,861. The cell line which produces BAT123 is on deposit at the ATCC under accession number HB 10438.

Suitable monoclonal Ab1, including BAT123, are produced by first immunizing an animal, preferably a mouse, with a suitable antigen. The antigen is usually a pathogen or allergen or a potential pathogen or allergen. The antigen can be in whole form, *e.g.*, whole HIV-1 virions. Cells infected with the virus and expressing the antigen or immunogenic domains of the virus can also be used. Specific viral proteins, such as the envelope glycoproteins, may be purified from the lysates of infected cells or viruses.

The immunogenic domains of the antigen themselves, or synthetic or recombinant peptides which have the same or an immunologically equivalent sequence to these immunogenic domains, can also be used. These synthetic or recombinant peptides for use in immunization can be synthesized by conventional techniques, such as with the RaMPS system (DuPont

DeNemours & Co.), which applies Fmoc chemistry. Alternatively, recombinant peptides containing these peptides may be biosynthesized by expressing in *E. coli* or eukaryotic cells the gene segments containing the appropriate coding sequences.

When using a synthetic peptide segment as an immunogen, it is usually more effective to conjugate it to a protein carrier, for example, HBsAg, hepatitis B virus core antigen, ovalbumin, bovine serum albumin, or preferably keyhole lympet hemocyanin ("KLH"). If the peptidic segment lacks a lysine residue or if the lysine residue is in the middle part of the segment, it is desirable to add a lysine residue at the C-terminal end. Because the N-terminus already has an α-amino group, the modified synthetic peptide will have two available amino groups for linking.

Multiple molecules of peptides can be conjugated to each molecule of the carrier to make the immunogen. With KLH, a preferred molar ratio for peptide/KLH is 10. The conjugation can be done with well established methods using glutaraldehyde or bis (sulfosuccinimidyl) suberate or preferably disulfosuccinimidyl tartrate as the cross-linkers.

A preferred immunization protocol for preparing the Ab1 monoclonal antibodies is to inject into each mouse 50 μg of the conjugate of KLH and the aforementioned recombinant or synthetic

peptides in Freund's complete adjuvant. Two and four weeks later, the same amount of antigen is given subcutaneously in Freund's incomplete adjuvant. After about six weeks, the fourth antigen injection is given intraperitoneally in saline. Mice are sacrificed 4 days after the last injection and the spleens (or sometimes the lymph nodes) are removed for preparing single cell suspensions for fusion with myeloma cells.

A similar protocol can be used for immunization with native viral antigens purified from virus-infected cells. One example of such a purified viral antigen is HIV-1 gp120. It can be isolated from the lysates of HIV-1-infected cells, such as the human T cell line, H9.

Lymphocytes from the spleens (or lymph nodes) which have been removed from the mice can be fused with myeloma cells to prepare hybridomas secreting the Ab1 monoclonal antibodies. The fusion procedure with polyethylene glycol and other various procedures concerning the cloning and the culturing of hybridomas have been well established. The preferred protocol is the well-known one described by Hudson, L. and Hay, F.C. (Practical Immunology, 2nd edition, pp. 303-313, 1980, Blackwell Publishing Co., Boston), in which the lymphocytes are fused with non-secreting mouse myeloma cells, such as NS-1 or Sp2/0 cells,

using polyethylene glycol.

The screening of hybridomas for monoclonal antibodies reactive with the immunogen can be performed with an enzyme linked immunosorbent assay (ELISA). A synthetic or recombinant peptide having the same sequence as a portion of the immunogen is used as the solid-phase antigen. A preferred solid phase antigen is the conjugate of such a synthetic or recombinant peptide with a carrier protein different from that used with the immunogen. An appropriate carrier protein can be bovine serum albumin or ovalbumin, provided they were not used as carriers in the immunization. Monoclonal antibodies (Ab1) specific for the immunogen will then be screened for specific binding to the intact native antigen, which, for example, can be done in this case by using immunofluorescence flow cytometric analyses of staining of HTLV-III_B-infected H9 cells.

After the Ab1 are isolated and characterized, they are used to immunize mice to create the Ab2. A similar protocol to that described for the Ab1 immunization can be used to create the Ab2. A particularly preferred protocol for this immunization, used in producing AB19-4 from BAT123, is to first conjugate Ab1 to KLH using glutaraldehyde as described by Maloney *et al.*, *Hybridoma* 4:191 (1985). Mice are then immunized intraperitoneally with 100

μg of the Ab1-KLH conjugate at one month intervals for three months. Three days after the final immunization, the mice are killed, and the spleen cells are isolated and fused with Sp2/0 myeloma cells to create the Ab2, using the fusion techniques set forth above. *See* U.S. Patent Application Serial No.07/454,161; Fung *et al., J. Immunol.* 145:2199-2206 (1990).

To make the anti-idiotype/carrier conjugate, the Ab2 are then linked to a suitable carrier. The carrier/anti-idiotype conjugate can then be administered as a vaccine for use in active immunization.

The carrier chosen is one which can enhance endogenous production of antibodies (Ab3), which can react with the original antigen which was used to produce Ab1. Preferably, the carrier is one the subject has previously been immunized with or to which he has otherwise been exposed.

The preferred carrier proteins for use with anti-idiotypes which induce Ab3 against HIV-1, such as AB19-4, are HBsAg and HIV-1 p24. Alternatively, the carrier can be a T helper peptide of HBsAg or HIV-1 p24. The reason that these carriers are preferred is explained in the Summary of the Invention. The Ab3 which are induced by such a carrier/anti-idiotype conjugate will bind to the original antigen, *i.e.*, the PND of gp120 of HIV-1, and can

neutralize HIV-1 or cause destruction of HIV-1 infected cells.

The T helper determinants of HIV-1 p24 are contained within amino acid residue numbers 213-227 (Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg), numbers 233-247 (Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile), numbers 263-277 (Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr), numbers 288-313 (Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val). See Mills, K.H. *et al.*, J. Immunol. 144:1677-1683 (1990); Chong, P. *et al.*, *FEBS Letters* 264:231-234 (1990). The T helper peptides of gp120 are at amino acid residue numbers 421-436 (T_1), having the sequence Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala, and at residue numbers 105-117 (T_2), having the sequence His Glu Asp Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala. T helper peptides having any of these sequences, or immunological equivalents of these sequences, are suitable for conjugation with AB19-4 or other anti-idiotypes which induce Ab3 against HIV-1.

HBsAg also contains determinants recognized by antigen-specific receptors on T helper cells. In mice, it was found that one such determinant is in the pre-S(1) region of HBsAg, amino acid residue numbers 12-32, having the sequence Met Gly Thr Asn

Leu Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Leu Asp Pro. The other determinant also in the mouse pre-S(1) region is from amino acid residue numbers 94-117, having the sequence Pro Ala Ser Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Ile Ser Pro Pro Leu Arg Asp Ser His Pro. *See* Milich, D.R. *et al., J. Immunol.* 4457-4465 (1987). Another mouse T cell determinant is in the pre-S(2) region of HBsAg, amino acid residue numbers 120-132, having the sequence Met Gln Trp Asn Ser Thr Ala Leu His Gln Ala Leu Gln. *See* Neurath *et al., Science* 224:392-395 (1984).

The existence of human T cell determinants in the S region of HBsAg has also been verified. Once such human T cell determinant is from amino acid residue numbers 4-33, having the sequence Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp (as determined from HBV strain adw). Celis, E. *et al., J. Immunol.* 140:1808-1815 (1988). Three other human T cell determinants are respectively from amino acid numbers 48-86, having the sequence Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Cys Leu Arg Arg Phe Ile, from amino acid residue numbers 38-52, having the sequence Ser

Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu Gly Pro Asn, and from amino acid residue numbers 110-136, having the sequence Ile Pro Gly Ser Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly Asn Ser Met Phe Pro Ser Cys (as determined from HBV strain adw). Milich, D.R. *et al., J. Immunol.* 134:4203-4211 (1985). T helper peptides having any of these sequences, or immunological equivalents of these sequences, are suitable carriers for conjugation with AB19-4 or other anti-idiotypes which induce Ab3 against HIV-1.

AB19-4 has some properties which make it particularly well-suited for use in a conjugate of the invention. AB19-4 has been shown to induce, in rabbits, production of Ab3 which neutralize HTLV-III_{MN} and HTLV-III_B. This result is somewhat unexpected because the Ab1 (BAT123) with which mice were immunized to produce AB19-4, neutralizes only HTLV-III_B. This result suggests that the paratope-specific anti-idiotype, Ab2, can broaden the reactivity in the immunized individuals so that related strains of HIV-1 are bound and neutralized.

A conjugate of AB19-4 and HBsAg, HIV-1 p24, or HIV-1 gp120, or a T helper peptide thereof, will therefore be useful for immunizing AIDS patients or asymptomatic individuals infected with these two HIV-1 strains, and who have been previously

exposed to HIV-1 p24 or HBsAg. The broadening neutralizing properties of antibodies induced by AB19-4 makes it likely that the conjugate of AB19-4 and these carriers will be useful in vaccinating against other related HIV-1 strains and isolates as well.

The anti-idiotypes first obtained, as noted above, will usually be murine-derived. Murine-derived anti-idiotypes will often induce endogenous antibodies against their Fc region, in addition to inducing the HIV-1 neutralizing Ab3. Production of endogenous antibodies to the Fc region is not necessary or desired. In fact, production of such antibodies against the Fc region could reduce the immunogenic effect of the anti-idiotype vaccine before it could function to induce production of Ab3.

Thus, the preferred anti-idiotypes for use in the conjugates of the invention have human constant regions. These include whole human anti-idiotypes, chimeric anti-idiotypes wherein the variable region is of murine origin and the constant region is of human origin, and anti-idiotypes wherein only the complementarity determining regions are of murine origin and the remainder of the variable regions, and the entire constant regions, are of human origin.

Such human (or humanized) anti-idiotypes, however, will be poorly immunogenic and will induce little Ab3. This reduced

immunogenicity results because the immunized subject will not have any T helper cells that can recognize the human constant regions, which are autologous.

In order to provide a determinant recognized by the antigen-specific receptors on helper T cells, it is preferable to conjugate the human (or humanized) anti-idiotypes to a carrier to which the recipient has been previously exposed. With such a conjugate, however, there is a possibility that the T cell help will cause an autoimmune antibody response against the human Fc constant regions. Thus, rather than conjugating a whole anti-idiotype to the carrier, it is more preferable that one conjugate the carrier with anti-idiotype $F(ab')_2$, Fab, or Fv fragments, none of which have a Fc portion.

The chimeric and humanized anti-idiotypes can be produced by transfecting non-producing mouse myeloma cells with the hybrid genomic DNA, or cDNA. If one is making a chimeric anti-idiotype, the hybrid genomic DNA or cDNA will contain the human constant regions and the mouse variable region. If one is making an anti-idiotype in which only the CDRs are of mouse origin, the hybrid genomic DNA or cDNA will contain human constant regions, mouse CDR regions, and the remainder of the variable regions will be human.

Human anti-idiotypes can be produced by using human expression libraries (*e.g.*, Stratagene Corp., La Jolla, California) to produce fragments of human anti-idiotypes (V_H, V_L, F_v, Fd, Fab, or $F(ab')_2$). Conjugates suitable for vaccination can be made from the anti-idiotype fragments Fab, $F(ab')_2$, or Fv, or one can use the fragments to construct whole human anti-idiotypes using techniques similar to those for producing chimeric anti-idiotypes.

One can also create single peptide chain anti-idiotypes. In such anti-idiotypes, the heavy and light chain F_v regions are connected. *See* Huston, J.S. *et al.*, Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988).

The conjugates of the invention are administered subcutaneously or intramuscularly. They can be administered together with appropriate adjuvants, such as threonyl muramyl dipeptide.

It is also noted that either of the conjugates which induce Ab3 against HIV-1 can be administered in conjunction with antibodies which effect passive immunization against HIV-1. For this application, HIV-1 neutralizing antibodies (which are preferably those Ab1 having idiotopes which are not recognized and neutralized by the Ab2 which is used concomitantly) are administered directly.

Passive immunization is likely to be most effective in a person previously exposed to HIV-1, but whose immune system is not yet seriously compromised. The neutralizing antibodies which are introduced will lower the viral titer. It may lower it to the point where remaining virus can be eliminated by the body's immune defense mechanisms. If neutralizing antibodies are administered in combination with a preferred conjugate of the invention, the conjugate will induce production of HIV-1 neutralizing antibodies which can exist for long periods.

Passive immunization is also likely to be useful for pregnant women who are HIV-1 infected. It is known that where the endogenous titer of neutralizing antibody against the principal neutralizing domain of gp120 of HIV-1 is relatively high, the child is less likely to be infected by the virus. *See* Devash, Y. *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3445-49 (1990). The total neutralizing antibody titer can be effectively raised by administering a combination of the preferred conjugate and neutralizing antibodies to HIV-1.

Experimental Verification of the Efficacy of the Conjugates of the Invention in Inducing Ab3 Production

Animal experiments can be used to determine whether the carrier/anti-idiotype conjugate is more effective than an anti-idiotype alone in inducing production of Ab3. In an exemplary

experiment, a first group of mice is injected with saline. A second group of mice is immunized with ovalbumin or another carrier protein other than HBsAg. A third group is immunized with HBsAg.

In the preferred immunization protocol, each of the second and third groups of mice is injected subcutaneously twice with 10 μg of the selected carrier, together with adjuvants. For the first injection, the adjuvant is Freund's complete adjuvant, and for the second, the adjuvant is Freund's incomplete adjuvant. The first group of mice receive injections of adjuvants alone, without the specific immunogen.

After an appropriate period passes for an immune response against the immunogens to occur, the control mice, the ovalbumin-immunized mice, and the HBsAg-immunized mice are immunized with an AB19-4/HBsAg conjugate in Freund's incomplete adjuvant. Those in the third group should yield the highest titer of Ab3 against HIV-1.

Additional control groups of mice can also be used. A fourth group, previously immunized with HBsAg, can be immunized with AB19-4 alone, and a fifth group, also previously immunized with HBsAg, can be immunized with a mixture (rather than a conjugate) of AB19-4 and HBsAg. The third group of mice

should also produce higher antibody titers than the fourth and fifth groups.

The same experimental design should also be repeated with a conjugate of AB19-4 and a T helper peptide of HBsAg. This T helper peptide is synthesized and conjugated to AB19-4 employing the methods described in this application. These mouse studies will verify that an anti-idiotype/HBsAg vaccine conjugate is more efficacious than AB19-4 alone, or than a mixture of AB19-4 and HBsAg, in inducing Ab3 production.

Equivalents

The terms, expressions and examples herein are exemplary only and not limiting, and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. All such equivalents are intended to be encompassed by the following claims.

What Is Claimed Is:

1. A vaccine for actively immunizing a mammal, comprising:

an anti-idiotype antibody conjugated to a carrier, wherein the carrier is one against which the vaccine recipient has previously been immunized or otherwise previously exposed or which enhances the immunogenic response to the anti-idiotype antibody.

2. The vaccine of claim 1 wherein the anti-idiotype antibody is a monoclonal antibody.

3. The vaccine of claim 2 wherein the anti-idiotype antibody induces production of neutralizing antibodies against HIV-1.

4. The vaccine of claim 1 wherein the carrier is a peptide including a determinant recognized by the antigen-specific receptors on T helper cells.

5. The vaccine of claim 1 wherein the carrier is HBsAg or HIV-1 p24.

6. The vaccine of claim 5 wherein the carrier is a peptide including determinants recognized by p24-specific receptors on T helper cells.

7. The vaccine of claim 6 wherein the peptide includes at least one of the following sequences, or an immunologically equivalent sequence:

Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln

Met Arg

Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile;

Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr;

Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys

Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val.

8. The vaccine of claim 5 wherein the carrier is a peptide including determinants recognized by HBsAg-specific receptors on T helper cells.

9. The vaccine of claim 8 wherein the peptide has one of the following sequences, or an immunologically equivalent sequence:

Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp;

Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Cys Leu Arg Arg Phe Ile;

Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu Gly Pro Asn;

Ile Pro Gly Ser Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly Asn Ser Met Phe Pro Ser Cys.

10. The vaccine of claim 1 wherein the carrier is an anti-*migis-δ* or an anti-*migis-μ* antibody, which respectively target the extracellular segments of membrane-bound IgD and membrane-bound IgM.

11. The vaccine of claim 1 wherein the carrier is a peptide including determinants recognized by gp120-specific receptors on T helper cells.

12. The vaccine of claim 11 wherein the peptide has one of the following sequences, or an immunologically equivalent sequence:

Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala;

His Glu Asp Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala.

13. The vaccine of claim 2 wherein the anti-idiotype antibody is AB19-4.

14. The vaccine of claim 2 wherein said monoclonal antibody is a chimeric antibody having animal variable regions and human constant regions.

15. The vaccine of claim 14 wherein said chimeric antibody has murine variable regions and human constant regions.

16. The vaccine of claim 15 wherein said monoclonal antibody is a human monoclonal antibody or a fragment thereof.

17. The vaccine of claim 14 wherein said monoclonal antibody has portions of its complementarity determining region derived from an animal and the other portions of its variable region, and its entire constant region, derived from a human.

18. The vaccine of claim 16 wherein the human monoclonal antibody fragment is Fab, $F(ab')_2$, or Fv.

19. A method of actively immunizing against a potential pathogen or allergen, comprising administering an effective amount of a vaccine, wherein the vaccine includes a monoclonal anti-idiotypic antibody specific for the pathogen or allergen conjugated with an carrier protein, the carrier protein being one against which the vaccine recipient has previously been immunized or to which the vaccine recipient has otherwise previously been exposed.

20. The method of claim 19 wherein the anti-idiotype antibody induces production of neutralizing antibodies against HIV-1.

21. The method of claim 20 further including administering an antibody which neutralizes HIV-1.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08853

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 39/395, 39/44
US CL : 424/85.8, 85.91

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	424/85.8, 85.91

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[6]

CAS, APS, MEDLINE

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y	AIDS, Volume 4, No. 2, issued February 1990, Wilks et. al., "Anti-CD4 antibodies and screening for anti-idiotypic antibodies to anti-CD4 antibodies in HIV-seropositive people", pages 113-118, see entire document.	3-6
Y	Journal of Molecular Recognit., Volume 1, No. 4, issued April 1989, Bost et. al., "Production of anti-idotypic antibodies by immunization with a pair of complementary peptides.", pages 179-183, see entire document.	3-21
X Y	Science, Volume 226, issued 14 December 1984, McNamara et. al., "Monoclonal idiotope Vaccine against Streptococcus Pneumoniae infection", pages 1325-1326, see entire document.	1-2 3-21
E,Y	US, A, 5,079,344 (Chang et. al.) 07 January 1992, See entire document.	1-21

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
25 FEBRUARY 1992	11 MAR 1992
International Searching Authority[1]	Signature of Authorized Officer[20]
ISA/US	Lila Feisee

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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Virology, Volume 174, No. 1, issued January 1990, Zhou et. al., "Administration of non-internal image monoclonal anti-idiotypic antibodies induces idiotype-restricted responses specific for human immunodeficiency virus envelope glycoprotein epitopes.", pages 9-17, see entire document.	1-21

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.